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MATERNAL ANTIBODY TRANSFER AND MENINGEAL WORM INFECTION RATES IN KENTUCKY ELK

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ABSTRACT OF THESIS

MATERNAL ANTIBODY TRANSFER AND MENINGEAL WORM INFECTION RATES IN KENTUCKY ELK

Elk (*Cervus elaphus*) were historically present throughout Kentucky, but were extirpated by the mid 19th century. Kentucky Department of Fish and Wildlife Resources initiated elk reintroduction efforts in 1997, resulting in a self-sustaining population. I designed this project to study the effects of a parasitic nematode, meningeal worm (*Parelaphostrongylus tenuis*), on Kentucky's elk herd. I examined potential maternal transfer of *P. tenuis* antibodies to elk calves, and investigated the relationship between elk habitat use and meningeal worm infection. I captured neonatal elk in 2004-06, fitted them with VHF transmitters, and collected blood samples for an enzyme-linked immunosorbent assay (ELISA) to determine *P. tenuis* infection. I monitored animals to determine habitat use, and attempted to recapture each individual to collect a follow-up blood sample. I found substantial rates of maternal meningeal worm antibody transfer (55%) over the course of the study. Neither sex nor predicted birth weight was associated with increased likelihood of obtaining maternal antibodies. Habitat variables associated with *P. tenuis* infection included herbaceous, shrub, and bare cover types, herbaceous mean core area, forest edge density, and forest mean core area. Confounding variables complicated habitat data analysis, but high rates of maternal *P. tenuis* antibody transmission suggested that meningeal worm infection does not threaten the long-term viability of the Kentucky elk herd.

KEYWORDS: Elk, Meningeal worm, *Parelaphostrongylus tenuis*, Kentucky, ELISA

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2/13/09

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THESIS

Willie Elwood Bowling

The Graduate School

University of Kentucky

2009

MATERNAL ANTIBODY TRANSFER AND MENINGEAL WORM INFECTION RATES IN KENTUCKY ELK

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture at the University of Kentucky

By

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Lexington, Kentucky

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2009

I dedicate this work to the people of eastern Kentucky – my people. Your unfailing support has always made it all worthwhile.

Also, to the legacy of Dr. Dave Maehr: may your legend never diminish.

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CHAPTER ONE

INTRODUCTION

Elk (*Cervus elaphus*) were widespread throughout the area now comprising the continental United States prior to European settlement, with anecdotal and archeological evidence suggesting that this species was the most widely distributed North American cervid (O'Gara and Dundas 2002). Elk were historically abundant in Kentucky, but unregulated hunting and habitat loss led to its statewide extirpation by 1850 (Funkhouser 1925, Barbour and Davis 1974). The United States Department of Agriculture Forest Service sponsored a small-scale reintroduction effort in 1996 to create a captive herd at Land Between the Lakes National Recreation Area in western Kentucky. The success and popularity of this project led the Kentucky Department of Fish and Wildlife Resources (KDFWR) to begin consideration of a larger-scale elk restoration of free-ranging individuals (Larkin et al. 2001). Subsequent public opinion meetings demonstrated that a majority of Kentucky citizens advocated elk restoration for additional hunting opportunities, increased potential for wildlife viewing, and development of an eco-tourism market (Maehr et al. 1999).

Even with widespread public support, the presence of intensive agricultural operations and established urban areas precluded the majority of Kentucky as socially suitable for elk reintroduction (Larkin et al. 2001). The paucity of commercial agriculture and relatively low human population density made southeastern Kentucky the only viable choice for elk reintroduction efforts in Kentucky (Larkin 2001). In addition to these factors, the region's mixed mosaic landscape of deciduous forests and large grassland patches created by surface mine reclamation provided herbaceous habitat for

elk (Larkin et al. 2001, Schneider et al. 2006). KDFWR translocated 1,541 elk from source populations in the western United States from 1997 - 2002 (Maehr et al. 1999, Seward 2003). Subsequent research conducted on this population included neonatal survival and habitat use (Seward 2003), relationships between elk, coyote (*Canis latrans*) and white-tailed deer (*Odocoileus virginianus*) (Cox 2003), demographics and spatial characteristics (Larkin 2001), meningeal worm (*Parelaphostrongylus tenuis*) and ectoparasite issues (Alexy 2004), activity, movement and habitat use (Wichrowski et al. 2006), and food habits (Schneider et al. 2006).

The KDFWR population model estimated the elk population at 8,500 in 2008 (T. Brunjes, Kentucky Department of Fish and Wildlife Resources, personal communication), the largest elk herd in eastern North America. Other eastern states (Arkansas, Michigan, Minnesota, North Carolina, Pennsylvania, Tennessee and Wisconsin) have conducted smaller-scale elk reintroduction projects and established free-ranging herds (O' Gara and Dundas 2002, D. Ledford, Rocky Mountain Elk Foundation, personal communication).

Twentieth century elk restoration efforts in eastern states were often unsuccessful. Although these failed attempts often lacked definitive scientific data regarding reasons for decline, managers often implicated meningeal worm infection (Carpenter et al. 1973, Anderson and Prestwood 1981, Raskevitz et al. 1991). *P. tenuis* is a parasitic nematode that can infect a wide variety of cervid species, including white-tailed deer, elk (Anderson et al. 1966), moose (*Alces alces*) (Thomas and Cahn 1932), caribou (*Rangifer tarandus*) (Trainer 1973), pronghorn antelope (*Antilocapra americana*) (Simmons et al. 2002), mule deer (*Odocoileus hemionus columbianus*) (Tyler and Hibler 1980), black-

tailed deer (*O. h. columbianus*) (Nettles et al. 1977), and fallow deer (*Dama dama*) (Pybus et al. 1992). White-tailed deer serve as the sole normal definitive host for *P. tenuis*, and are generally not pathologically affected by meningeal worm infections (Forrester and Lankester 1998). *P. tenuis* infection in abnormal definitive hosts often causes severe neurological trauma that can compromise an individual's fitness (Anderson 1966, Garner and Porter 1991, Pybus et al. 1992).

The most prevalent explanation for the differential outcomes exhibited in *P. tenuis* infection of cervids stems from parasite ecology, which posits that parasites often co-evolve with a specific host but remain capable of infecting species with a related evolutionary lineage (Parsons 1983, Aguirre and Starkey 1994). Although Eisenberg (1987) contends that cervid evolutionary history is often vague, it is generally accepted that white-tailed deer and elk lineages split from a shared Miocene ancestor (Scott and Janis 1987, Pitra et al. 2004). Phylogenetic evidence suggests that elk and white-tailed deer were geographically separate throughout much of their evolution, with the Cervinae subfamily located in Eurasia while the Odocoileinae subfamily developed in North America (Scott and Janis 1987). The two groups ostensibly had no contact until elk crossed the land bridge from Eurasia into North America in the early Pleistocene (Lundelius et al. 1987). During the previous geographic isolation, *P. tenuis* had colonized white-tailed deer, but the phylogenetic relationship between white-tailed deer and elk was close enough to permit meningeal worm infection of both species (Platt 1984, Carreno and Lankester 1994).

The ensuing relationship among *P. tenuis*, white-tailed deer, and elk has been regarded as a classic case of parasite-mediated competition (Price et al. 1988, Schmitz

and Nudds 1994). Parasite-mediated competition occurs when two or more species share a common parasite, but the parasite differentially diminishes competitive ability of one or more species (Price et al. 1986). Upon colonizing North America, elk likely posed competition to white-tailed deer for food resources. Given that elk are larger and can better exploit foods of lesser digestibility than white-tailed deer (Cook 2002), it would appear that elk could exclude white-tailed deer from many food resources. Meningeal worm infection typically causes a negative response in abnormal hosts, and may have permitted white-tailed deer to compete more effectively with elk than would have otherwise been possible following Nearctic colonization by the larger cervids (Barbehenn 1969, Price et al. 1988, Aguirre and Starkey 1994, Schmitz and Nudds 1994). Thus, the prejudicial immune challenge posed by *P. tenuis* may have amounted to "a powerful competitive weapon (Haldane 1949)" that favored white-tailed deer by reducing elk densities across their shared range.

P. tenuis, like all members of the Protostrongylidae family, requires both intermediate and definitive hosts (Adamson 1986). In white-tailed deer, adult *P. tenuis* most often occurs in the cranial meninges. Female meningeal worms release eggs into venous circulation, where they eventually lodge in alveolar capillaries (Lankester 2001). Eggs develop into first stage larvae (L_1) within the lungs, eventually migrating through the respiratory tree into the pharynx. At this time the larvae are swallowed, passed through the digestive tract, and vacated with the feces (Anderson 1963). Terrestrial gastropods contact L_1 larvae on the surface of feces or the soil matrix, allowing the larvae to infect the gastropod by penetrating the foot (Anderson 1963). First stage larvae then undergo molting to second stage larvae (L_2), and then the third stage larvae (L_3). At this

point, the larvae are infective (Anderson 1963). If a cervid ingests an infected gastropod during the course of feeding, the L_3 penetrates the gastrointestinal wall during digestion and migrates through lateral spinal nerves into the spinal cord (Anderson 1965, Anderson and Strelive 1967). Larvae molt twice more to enter the sub-adult stage, upon which they move through the spinal subdural space to the cranium. Maturation is completed in the cranial meninges (Anderson 1965, Anderson 1968).

Third stage *P. tenuis* larvae that infect elk exhibit similar tissue migration patterns as noted in white-tailed deer, but tend to have greater residence time in the spinal cord of elk as compared to white-tailed deer (Anderson et al. 1966). In addition to observing *P. tenuis* spending increased time in the spinal cord of elk, Anderson et al. (1966) also noted that in the abnormal host *P. tenuis* tended to coil upon itself, producing neural trauma that likely results in the motor skill disruptions associated with meningeal worm infection. While elk are not *P. tenuis*' normal definitive host, meningeal worms can complete their life cycle and achieve reproductive success using the larger cervid (Anderson et al. 1966, Pybus et al. 1989).

Despite the ability of meningeal worms to perpetuate themselves through a variety of hosts, no records exist of *P. tenuis* west of 105 degrees west longitude (Thorne et al. 2002). Suitable intermediate and definitive hosts are found beyond the meningeal worm's presumed western boundary (Karlin 1961, Kralka 1986, Samuel et al. 1992), but Anderson (1972) suggested that the prairie biome of interior North America poses an ecological barrier to *P. tenuis* expansion due to desiccation of the intermediate host. While an authoritative explanation for *P. tenuis*' range has proven elusive, research has indicated that meningeal worm is common throughout most of eastern North America

(Prestwood and Smith 1969, Comer et al. 1991, Wasel et al. 2003). This prevalence has posed challenges to wildlife managers seeking to reintroduce extirpated cervids considered abnormal hosts for *P. tenuis* (Carpenter et al. 1973, Severinghaus and Darrow 1976, Bender et al. 2005).

P. tenuis is widespread in Kentucky (Prestwood and Smith 1969, Comer et al. 1991, Larkin et al. 2003*a*), and unsurprisingly was identified as a causal factor of elk mortalities in the restored herd (Larkin 2003*a*, Alexy 2004). Samuel et al. (1992) demonstrated that elk mortality was positively correlated with the number of *P. tenuis* larvae ingested, and Alexy (2004) noted significant infection rates in intermediate hosts throughout the Kentucky elk restoration zone.

Some studies have suggested that individuals within cervid populations may develop an immune response to meningeal worm infection that may protect them from subsequent fatal *P. tenuis* infections (Slomke et al. 1995, Bienk et al. 1998). Confirmation of such a protective mechanism could help elucidate this host-parasite relationship (Welch et al. 1991), but verification was not possible until development of an enzyme-linked immunoassay (ELISA) to identify *P. tenuis* antibodies in blood samples (Ogunremi et al. 1999, Ogunremi et al. 2002*b*). ELISA methods offer benefits over traditional meningeal worm research techniques, including increased accuracy in determining infection (Welch et al. 1991, McCollough and Pollard 1993, Forrester and Lankester 1997) and the ability to detect clinically silent infections (Ogunremi et al. 2002*a*).

The objective of my research was to examine the relationship between elk habitat use and meningeal worm infection in Kentucky. I sought to explore the roles of maternal

antibody transfer, sex, cohort year, and habitat use in relation to *P. tenuis* immune response. Habitat use data may also permit the creation of a model describing the most important habitat factors associated with meningeal worm infection in Kentucky.

Hypotheses

- Elk neonates can acquire *P. tenuis* antibodies through maternal immunoglobin transfer.
- Habitat characteristics shown to have higher presence of infected intermediate hosts will be associated with greater *P. tenuis* antibody levels in elk.

CHAPTER TWO

MATERNAL TRANSFER OF MENINGEAL WORM ANTIBODIES

Introduction

When a pregnant mammal is immunologically challenged, the resulting antibodies can be passed to her offspring (Brambell 1970). Maternal immunoglobin transfer is influenced by genetic propensity of mother and offspring, environmental factors, and disease prevalence during gestation (Gray 1970, Grindstaff et al. 2003). Transfer of maternal antibodies to progeny has long been recognized in ruminant species (Famulener 1912, Reymann 1920). Various studies have concluded that maternal antibody transfer can benefit offspring (Grindstaff et al. 2003, Boulinier and Staszewski 2007) through enhanced immune function (Yasuda et al. 1998, Lemke and Lange 1999), improved growth rates (Robison et al. 1988), and increased survival following disease incidents (Zhang et al. 1988).

Parelaphostrongylus tenuis, or meningeal worm, is a parasitic nematode with several distinct life stages (Adamson 1986). The normal definitive host for *P. tenuis* is white-tailed deer (*Odocoileus virginanus*). In this relationship, adult meningeal worms produce larvae that are voided with the deer's feces (Anderson 1963, Lankester 2001). A variety of terrestrial gastropods species serve as the meningeal worm's intermediate host and are presumably infected after contacting the larvae on feces or the soil matrix (Anderson 1963, Lankester and Anderson 1968). If a white-tailed deer ingests an infected gastropod during the course of feeding, the larva migrates from the stomach during digestion, eventually reaching the cranium, where development to maturity occurs

within the cranial meninges (Anderson 1965, Anderson and Strelive 1967, Anderson 1968).

Meningeal worm infections are generally clinically benign in white-tailed deer (Forrester and Lankester 1998), but often harm abnormal hosts (Anderson 1966, Garner and Porter 1991, Pybus et al. 1992). Abnormal hosts include a wide variety of cervid species, including elk, (Anderson et al. 1966, Prestwood and Smith 1969), moose (*Alces alces*) (Thomas and Cahn 1932), caribou (*Rangifer tarandus*) (Trainer 1973), pronghorn antelope (*Antilocapra americana*) (Simmons et al. 2002), mule deer (*Odocoileus hemionus columbianus*) (Tyler and Hibler 1980), black-tailed deer (*O. h. columbianus*) (Nettles et al. 1977), and fallow deer (*Dama dama*) (Pybus et al. 1992). Clinical signs of meningeal worm infections in elk include adipsia, ataxia, visual impairment, seclusion from the herd, listlessness, and excessive salivation (Carpenter et al. 1973, Olsen and Woolf 1978). Anderson (1968) suggested that abnormal hosts undergo these neurological disorders because, in comparison to white-tailed deer, *P. tenuis* larvae reach abnormal hosts central nervous system in comparatively high numbers and spend more time developing within the spinal column.

P. tenuis commonly occurs throughout eastern North America, and is widespread in Kentucky (Prestwood and Smith 1969, Comer et al. 1991, Larkin et al. 2003*a*, Wasel et al. 2003). Larkin et al. (2003*a*) and Alexy (2004) identified *P. tenuis* infection as a causal factor of mortality in Kentucky's restored elk population, which was consistent with records of previous elk reintroductions in the meningeal worm's range (Carpenter et al. 1973, Severinghaus and Darrow 1976). Furthermore, Alexy (2004) reported significant *P. tenuis* infection in normal definitive and intermediate hosts throughout the

Kentucky elk restoration zone, suggesting that elk had a high likelihood of exposure to the parasite.

P. tenuis infection does not necessarily result in the death of the abnormal host (Samuel et al. 1992). Abnormal hosts have a higher incidence of debilitating effects associated with meningeal worm infection than do white-tailed deer, although cervid species – including elk – can be slightly refractory to *P. tenuis* infection (Olsen and Woolf 1979, Davidson et al. 1985, Lankester 2001). Larkin et al. (2003*a*) noted that most meningeal worm associated deaths occurred in animals < 3 years of age in the Kentucky elk population. This could potentially be a sign of increasing resistance to the parasite with age, as Colditz et al. (1996) asserted that immunological competence increased with age. Although Kentucky elk have a high probability of escaping fatal meningeal worm infections if they reach adulthood (Larkin 2003*a*, Alexy 2004), the effect of this parasite on population growth is unknown. Juvenile survival can enhance cervid population growth and colonization potential (Caughley 1977, Taber et al. 1982). As such, high observed rates of meningeal worm mortalities in younger age classes may negatively impact future population growth (Olsen and Woolf 1978, Larkin et al. 2003*a*, Larkin et al. 2003*b*). Potential detrimental effects of *P. tenuis* infection on juvenile elk may be reduced if immunologically naïve age classes obtain a degree of parasite resistance that aids survival until a complete immune response can be achieved.

One scenario of acquired resistance could come from acquisition of *P. tenuis* antibodies through passive maternal transfer. This mechanism of antibody transmission could protect juvenile elk from fatal parasite infection until they reach immunological maturity (Hattel et al. 2007), but maternal *P. tenuis* antibody transfer has not been

documented. I attempted to determine if elk calves can acquire *P. tenuis* antibodies through maternal immunoglobin transfer.

Study Area

The elk restoration zone (Figure 2.1) was originally comprised of 14 counties in southeastern Kentucky, but was expanded to 16 counties to facilitate potential movement between Kentucky and Tennessee elk populations (D. Crank, KDFWR, personal communication). This restoration zone lies along the Cumberland Plateau, an area historically characterized by rugged topography covered with continuous second and third growth deciduous forest (Overstreet 1984). Mixed-mesophytic forest association covered most of the mesic sites and richer soils, while oak-pine and oak-hickory associations predominated on more xeric locations (Wharton and Barbour 1973). Common overstory species throughout the region include yellow poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), red maple (*A. rubrum*), American beech (*Fagus grandifolia*), basswood (*Tilia* spp.), yellow buckeye (*Aesculus flava*), northern red oak (*Quercus rubra*), white oak (*Q. alba*), hemlock (*Tsuga canadensis*), black walnut (*Juglans nigra*), black cherry (*Prunus serotina*), shagbark hickory (*Carya ovata*), and white ash (*Fraxinus americana*) (Wharton and Barbour 1973).

Land use has considerably altered the eastern Kentucky landscape (Maehr et al. 1999), resulting in a mosaic that was approximately 80% second and third growth forest, 10% active and reclaimed surface mines, 9% agricultural or cleared lands, and 1% urban (Cox 2003). Species commonly found on reclaimed surface mines included Kentucky-31 tall fescue (*Lolium arundinaceum*), bush clover (*Lespedeza* spp.), birds-foot trefoil (*Lotus corniculatus*), crown vetch (*Coronilla varia*), perennial ryegrass (*Lolium perenne*),

orchardgrass (*Dactylis glomerata*), black alder (*Alnus glutinosa*), autumn-olive (*Elaeagnus umbellata*), white pine (*Pinus strobus*), and black locust (*Robinia psuedoacacia*) (Larkin 2001).

Hill (1976) described the climate as temperate humid continental, with warm summers and cool winters. Mean annual temperature was 13º C (National Oceanic and Atmospheric Administration 2007), and averaged 117 freeze-free days per year (United States Department of Agriculture Soil Conservation Service 1981). Precipitation averaged 125 cm annually, and was evenly distributed throughout the year (National Oceanic and Atmospheric Administration 2007).

Methods

Calf capture.— I captured elk neonates using three different search methods: monitoring cows for parturition behavior, ground-based field searches in traditional calving locations, and aerial searches using a helicopter equipped with an observer using an infrared scope. Calf capture occurred during the months of May and June from 2004-06. Capture techniques followed practices described by White et al. (1972) and Seward (2003).

I considered altered movement patterns, abrupt movement from the herd (Vore et al. 1996, Vore and Schmidt 2001), and a reluctance of lone females to move away in the presence of researchers (Seward 2003) as indicators of parturition behavior. Upon observing any of the aforementioned behaviors we proceeded to walk in a grid pattern throughout the general area in which we viewed the cow. I adjusted search pattern intensity to habitat type and relative cover, spending more time in areas with higher

amounts of vegetative cover. I continued in this manner until I either located the calf or decided that I had covered the area sufficiently to locate a calf had one been present.

I identified traditional calving locations through correspondence with Kentucky Department of Fish and Wildlife Resources personnel (K. J. Alexy, D. Crank, and C. Logsdon, KDFWR, personal communication). Calving areas frequently included forest edge, shrub habitat, or pond edges. I conducted ground searches in these areas using the technique described for parturition behavior searches.

I used a Thermal Eye 250D (L-3 Communications Infrared Products, Dallas, TX, USA) handheld infrared scope to locate bedded calves during aerial capture exercises. Helicopters employed in the aerial searches included a 206B Jet Ranger III (Bell Helicopter Textron, Inc., Fort Worth, TX, USA) and an Enstrom 280fx (Enstrom Helicopter Corporation, Menominee, MI, USA). After locating a calf, the observer in the helicopter directed capture efforts to a ground crew via a two-way radio.

I equipped captured elk with expandable VHF radio collars (Advanced Telemetry Systems, Inc., Isanti, MN., USA) that were equipped with a 4-hour, mortality delay switch. I placed one-piece plastic ear tags (Farnam Companies, Inc., Phoenix, AZ, USA) in each calf to facilitate recognition of individuals during tracking efforts. I collected approximately 20 mL of blood from the jugular vein of each calf. I also recorded neonate sex, approximate age based on umbilicus healing and hoof epithelium wear, Global Positioning System (GPS) location of the capture site, and body weight.

Blood sample analysis.— I separated whole blood into serum and red blood cells using a centrifuge, then stored the serum in cryogenic vials at -23º C for subsequent analysis. Prairie Diagnostic Services (Regina, Saskatchewan, Canada) conducted all *P.*

tenuis serodiagnostic tests using indirect ELISAs after Ogunremi et al. (2002*b*). Prairie Diagnostic Services designated serum samples as negative, suspect or positive with respect to *P. tenuis* antibody presence based on optical density values. Cutoff values for serum diagnosis were -0.150-0.399 for negative diagnosis, 0.400-0.699 for suspect diagnosis, and 0.700-1.687 for positive diagnosis. All suspect and positive diagnoses fell above the optical density value cutoff for positive diagnosis established by Ogunremi et al. (2002*b*). We considered all suspect diagnoses to be positive.

Data analysis.— Using the approximate age assigned to each calf at time of capture, I removed all calves >14 days old from the dataset to minimize the possibility of analyzing individuals that had already initiated an autonomous immune response. I chose 14 days as the cutoff point because elk calves rely primarily on their mother's milk for sustenance during the first two weeks of life, and consequently ingest little vegetation during this period (Cook 2002). Because foraging activity during the first 14 days of life is negligible, the probability that elk neonates ingest infected gastropods during this time is minimized. Furthermore, Ogunremi et al. (2002*b*) failed to detect *P. tenuis* antibodies prior to 14 days post-inoculation after administering infective meningeal worm larvae to previously uninfected elk.

I used Fisher's exact test (PROC FREQ; SAS Institute, Inc. 1998) to determine if calf sex affected acquisition of *P. tenuis* antibodies from maternal transfer (Appendix A) ($P \leq 0.05$). I performed logistic regression (PROC LOGISTIC; SAS Institute, Inc. 1998) using forward selection to determine if predicted calf birth weight was associated with *P. tenuis* antibody presence (Appendix B) ($P \leq 0.15$). I calculated predicted birth weight of neonates by multiplying estimated age (days) by 0.635 kg/100 kcal assumed daily growth

rate (Table 2.1; Cook et al. 1996, Seward 2003). I analyzed cohorts separately in all tests to circumvent possible annual differences in prevalence of *P. tenuis*.

Results

Maternal transfer of *P. tenuis* antibodies occurred in over half of elk neonates. I detected *P. tenuis* antibodies in 10 of 19 (53%) neonates in the 2004 cohort, 22 of 40 (55%) neonates in the 2005 cohort, and 21 of 38 (55%) neonates in the 2006 cohort (Table 2.2). Sex did not influence antibody transfer in 2004-06, so I pooled the data across all years ($P = 0.148$). Likewise, predicted birth weight was not significant to *P. tenuis* antibody presence in 2004-06, so I pooled data across all years ($P = 0.951$).

Discussion

Maternal transfer of P. tenuis antibodies.— Passive immunity was previously described in cervid species (Grimstad et al. 1987, Gaydos et al. 2002), but this study is the first to demonstrate maternal transfer of *P. tenuis* antibodies in elk. I observed *P. tenuis* antibody presence in over half of elk neonates in all three years of this study, which suggests that passive maternal transfer of anti-meningeal worm immunoglobins commonly occurs in Kentucky.

Sex and maternal transfer of P. tenuis antibodies.— Ruminants acquire maternal antibodies through ingestion of colostrum within hours of birth (Carpenter 1956, Baintner 2007), suggesting that any adaptive value inherent to antibody transfer operates regardless of sex. In this study, sex did not influence the presence of meningeal worm antibodies in elk calves. Previous studies have also demonstrated that the sex of the definitive host does not influence immune response to meningeal worm infection in adult animals (Thurston and Strout 1978, Olsen and Woolf 1979, Garner and Porter 1991).

Birth weight and P. tenuis antibody acquisition.—Roulin and Heeb (1999) proposed that antibody production is positively correlated with nutritional intake, while ungulate birth weight is directly affected by maternal dietary success during gestation (Blaxter and Hamilton 1980, Thorne et al. 1976, Keech et al. 2000). Accordingly, I expected higher predicted birth weights to be positively associated with prevalence of *P. tenuis* antibodies in elk calves, but I failed to detect any association.

One potential explanation for this result stems from my methodology. The calf aging process was subjective, and was conducted by several different individuals over the course of the project. Since it was not possible to quantify the potential error inherent to this process, I was unable to determine the accuracy with which birth weights were predicted. Therefore, there is a chance that I failed to detect an association due to sampling error.

Also, it is possible that the mothers of all study animals received adequate nutrition during gestation, permitting production of large calves while maintaining optimal immune performance. Seward (2003) concluded that Kentucky elk calves had relatively high birth weights compared to other populations, which he attributed to superior nutritional intake (Larkin et al. 2003*b*). Consequently, cow elk in this study could have obtained sufficient resources during gestation to produce large calves while sustaining production of *P. tenuis* antibodies.

Finally, differing genetic propensities within the population could have been more important than maternal nutritional intake for addressing *P. tenuis* infection. Boulinier and Staszewski (2007) suggested that genotypic variation influenced both maternal antibody production and efficacy of immunoglobin uptake by the offspring. This could

have allowed some cows to form stronger immune responses to *P. tenuis* infection, increasing the likelihood that her calf could obtain meningeal worm antibodies. Similarly, genetics likely provided some calves with greater inherent potential to absorb maternal *P. tenuis* antibodies.

Potential adaptive value of P. tenuis antibody acquisition.— Exposure of elk calves to maternal *P. tenuis* antibodies could favorably influence individuals in several ways. Passive immunity to the parasite could prevent infection while the juvenile elk is allocating resources to growth and development, potentially increasing fitness later in life (Gustafsson et al. 1994, Buechler et al. 2002). Early exposure to meningeal worm antibodies could challenge the neonate's immune system, thus increasing the potential for a stronger, secondary immune response should the individual come into contact with infective *P. tenuis* larvae later in life (Grindstaff et al. 2003, Boulinier and Staszewski 2007).

Alexy (2004) noted that calves and yearlings accounted for 80% of the *P. tenuis*-related deaths in the Kentucky elk herd. Of the 62 study animals that I monitored from birth to 2 years of age, I recorded only one death (1.6%) from probable meningeal worm infection. The low observed mortality rate in this highly vulnerable age class suggests *P. tenuis* infection poses little threat to growth and viability of elk in this region. I did not have sufficient data to statistically determine if acquisition of maternal antibodies was associated with decreased likelihood of *P. tenuis*-related mortality, but neonate acquisition of meningeal worm antibodies may have conferred fitness advantages unapparent in my data.

Maternal reproductive significance.— In this study, I found that more than half of elk neonates acquired *P. tenuis* antibodies through maternal transfer. Antibody transmission from females to their progeny will not occur unless the dam has been exposed to the pathogen and mounted an immune response (Brambell 1969, Lemke et al. 2003). Grindstaff et al. (2006) suggested that maintenance of an elevated immune response required for transfer of maternal antibodies may decrease overall reproductive ability, but my findings illustrate that a substantial percentage of cow elk in Kentucky successfully birthed calves while maintaining *P. tenuis* antibody production. Given this information, it seems unlikely that meningeal worm infection diminished the reproductive potential of Kentucky elk over the course of this study.

Larkin et al. (2003*a*) cautioned that meningeal worm infection may limit the long-term viability of the Kentucky elk population, but my results do not support this assertion. Low observed *P. tenuis*-related mortality and evidence that previously infected female elk attained reproductive success suggest that meningeal worm infection does not pose a serious threat to immediate population growth or the long-term viability of the Kentucky elk population. KDFWR should continue periodic monitoring efforts to identify potential changes in herd demographics over time.

Future research could examine potential differences in meningeal worm infection survival among elk calves based on the acquisition of maternal *P. tenuis* antibodies. Such an investigation would necessitate high numbers of study animals to ensure observation of mortality rates sufficient for statistical testing. Research to determine the residence time of maternal *P. tenuis* antibodies in elk neonates could also impart valuable information about the potential adaptive value of meningeal worm antibody transfer.

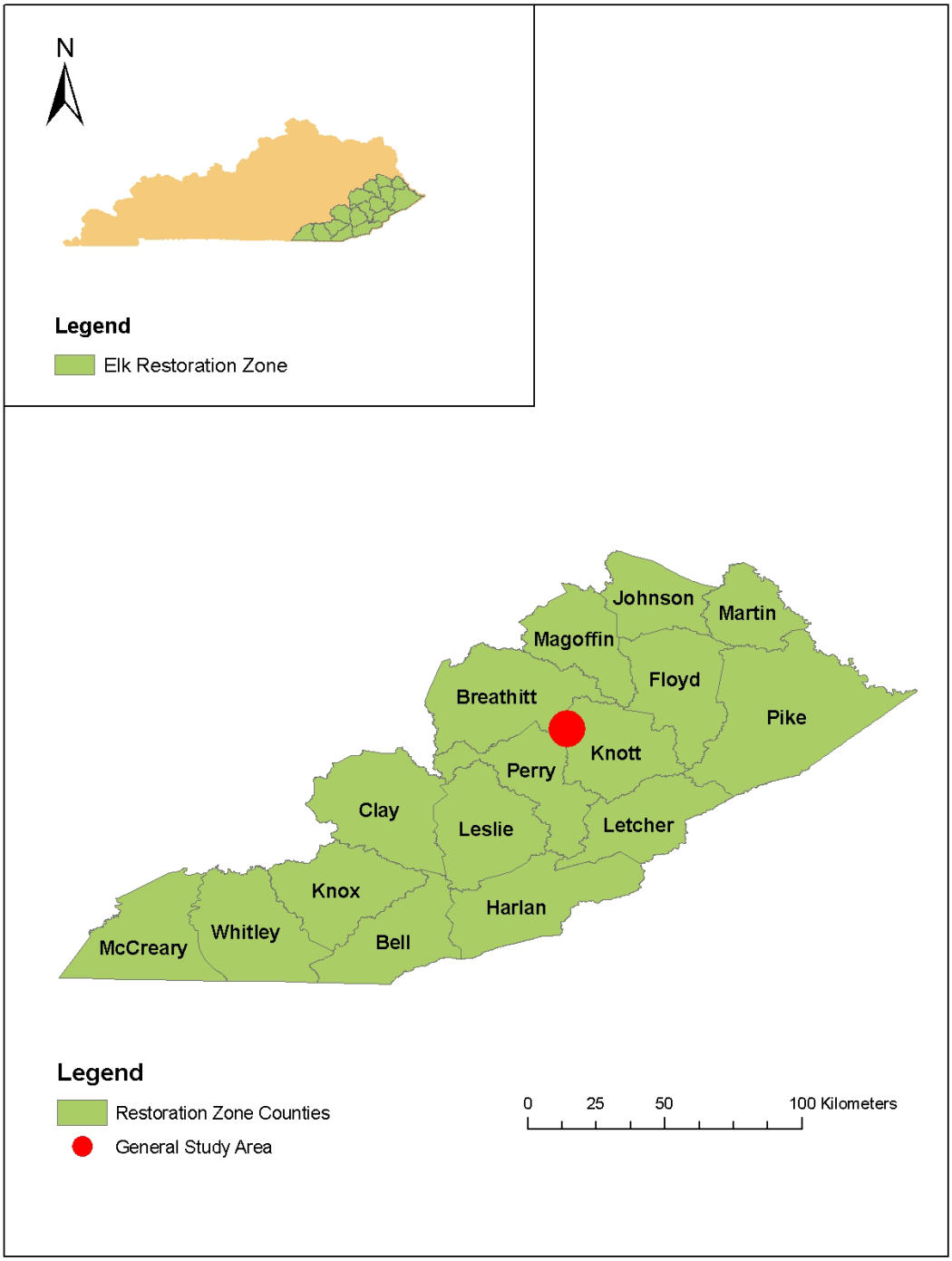

Figure 2.1. Elk restoration zone counties and general study area in relation to southeastern Kentucky, 2004-08.

Table 2.1. Predicted birth weights and *P. tenuis* antibody prevalence of Kentucky-born elk calves ≤ 14 days old, 2004-2006.

Year	ID	Sex	Estimated age (days) at capture	Capture weight (kg)	Predicted weight gain (kg)	Predicted birth weight (kg)	*P. tenuis* antibody presence
2004	C 8	F	6	27.2	3.8	23.4	+
	NT 1	F	7	24.5	4.5	20.0	+
	NT 3	F	3	20.2	1.9	18.3	+
	NT 4	F	3	19.3	1.9	17.4	+
	NT 6	F	1	18.1	0.6	17.5	+
	NT 7	F	4	21.8	2.5	19.3	-
	NT 8	F	11	29.5	7.0	22.5	-
	NT 9	F	3	15.9	1.9	14.0	-
	NT 10	F	6	21.3	3.8	17.5	-
	NT 14	F	1	17.0	0.6	16.4	+
	C 1	M	3	18.1	1.9	16.2	+
	C 3	M	1	18.6	0.6	18.0	+
	C 7	M	1	15.4	0.6	14.8	-
	C 11	M	6	19.1	3.8	15.3	-
	C 14	M	10	27.2	6.4	20.8	+
	NT 5	M	3	18.8	1.9	16.9	+
	NT 11	M	5	25.0	3.2	21.8	-
	NT 12	M	2	18.8	1.3	17.5	-
	NT 13	M	3	17.2	1.9	15.3	-
Mean			4	20.7	2.6	18.1	
2005	G 1	F	5	29.5	3.2	26.3	+
	G 3	F	10	26.3	6.4	19.9	-
	G 4	F	2	14.5	1.3	13.2	+

Table 2.1. (Continued)

Year	ID	Sex	Estimated age (days) at capture	Capture weight (kg)	Predicted weight gain (kg)	Predicted birth weight (kg)	*P. tenuis* antibody presence
2005	G 6	F	3	13.6	1.9	11.7	-
	G 8	F	4	17.2	2.5	14.7	+
	G 9	F	4	16.8	2.5	14.3	+
	G 10	F	6	20.4	3.8	16.6	+
	G 11	F	2	13.6	1.3	12.3	-
	G 13	F	4	17.7	2.5	15.2	+
	G 14	F	3	19.1	1.9	17.2	+
	G 15	F	6	23.6	3.8	19.8	-
	G 19	F	3	20.0	1.9	18.1	+
	G 22	F	2	13.6	1.3	12.3	+
	G 26	F	5	20.4	3.2	17.2	+
	G 29	F	4	20.4	2.5	17.9	+
	G 33	F	10	28.1	6.4	21.7	-
	G 34	F	4	20.0	2.5	17.5	-
	G 35	F	10	32.2	6.4	25.8	-
	G 36	F	5	21.8	3.2	18.6	-
	G 41	F	9	25.4	5.7	19.7	+
	G 44	F	4	15.9	2.5	13.4	-
	G 2	M	5	18.1	3.2	14.9	-
	G 5	M	4	20.9	2.5	18.4	+
	G 7	M	1	19.5	0.6	18.9	+
	G 12	M	5	21.8	3.2	18.6	-
	G 16	M	2	21.8	1.3	20.5	-
	G 17	M	7	28.1	4.5	23.6	+
	G 18	M	7	25.9	4.5	21.4	-

Table 2.1. (Continued)

Year	ID	Sex	Estimated age (days) at capture	Capture weight (kg)	Predicted weight gain (kg)	Predicted birth weight (kg)	*P. tenuis* antibody presence
2005	G 20	M	8	28.1	5.1	23.0	-
	G 21	M	9	28.1	5.7	22.4	-
	G 23	M	3	17.2	1.9	15.3	+
	G 24	M	4	16.3	2.5	13.8	+
	G 25	M	5	27.2	3.2	24.0	+
	G 27	M	8	29.5	5.1	24.4	-
	G 28	M	5	20.4	3.2	17.2	-
	G 30	M	10	34.9	6.4	28.5	-
	G 31	M	4	20.4	2.5	17.9	+
	G 32	M	4	20.4	2.5	17.9	+
	G 38	M	5	25.4	3.2	22.2	+
	G 39	M	8	25.4	5.1	20.3	+
Mean			5	21.8	3.3	18.5	
2006	B 2	F	5	21.3	3.2	18.1	-
	B 3	F	8	21.3	5.1	16.2	-
	B 6	F	8	27.2	5.1	22.1	-
	B 7	F	4	18.6	2.5	16.1	+
	B 8	F	6	24.0	3.8	20.2	-
	B 9	F	12	31.8	7.6	24.2	+
	B 10	F	7	20.4	4.4	16.0	-
	B 13	F	7	20.9	4.4	16.5	-
	B 15	F	4	16.3	2.5	13.8	-
	B 16	F	14	32.2	8.9	23.3	+
	B 19	F	4	20.0	2.5	17.5	+
	B 20	F	3	20.4	1.9	18.5	-

Table 2.1 (Continued)

Year	ID	Sex	Estimated age (days) at capture	Capture weight (kg)	Predicted weight gain (kg)	Predicted birth weight (kg)	*P. tenuis* antibody presence
2006	B 22	F	2	19.1	1.3	17.8	+
	B 23	F	6	23.1	3.8	19.3	-
	B 25	F	3	17.2	1.9	15.3	+
	B 29	F	2	14.5	1.3	13.2	-
	B 32	F	10	25.4	6.4	19.0	-
	B 33	F	7	22.7	4.4	18.3	+
	B 37	F	7	25.4	4.4	21.0	-
	B 40	F	2	16.3	1.3	15.0	+
	B 41	F	7	21.8	4.4	17.3	+
	B 42	F	9	28.1	5.7	22.4	+
	B 4	M	7	21.3	4.4	16.9	-
	B 11	M	5	17.7	3.2	14.5	+
	B 12	M	6	18.1	3.8	14.3	+
	B 17	M	3	21.3	1.9	19.4	+
	B 18	M	2	17.7	1.3	16.4	+
	B 21	M	8	25.4	5.1	20.3	+
	B 24	M	10	28.6	6.4	22.2	+
	B 26	M	4	21.3	2.5	18.8	+
	B 27	M	7	22.7	4.4	18.3	+
	B 30	M	6	20.0	3.8	16.2	-
	B 31	M	3	23.6	1.9	21.7	+
	B 34	M	13	25.9	8.3	17.6	+
	B 35	M	6	19.1	3.8	15.3	-
	B 36	M	3	19.5	1.9	17.6	+

Table 2.1 (Continued)

Year	ID	Sex	Estimated age (days) at capture	Capture weight (kg)	Predicted weight gain (kg)	Predicted birth weight (kg)	*P. tenuis* antibody presence
	B 38	M	4	21.3	2.5	18.8	-
	B 43	M	12	28.1	7.6	20.5	-
Mean			6	21.8	3.7	17.9	
Total			5	21.6	3.4	18.2	

Table 2.2. Prevalence of *P. tenuis* antibodies in Kentucky-born elk calves ≤ 14 days old as a function of sex and cohort, 2004-2006.

Year	Sex	No. calves tested	Percentage of calves exhibiting *P. tenuis* antibodies
2004	M	9	44
	F	10	60
	Both	19	53
2005	M	19	53
	F	21	57
	Both	40	55
2006	M	16	69
	F	22	45
	Both	38	55
Total	M	44	57
	F	53	53
Mean	Total	97	55

CHAPTER THREE

HABITAT USE AND PRESENCE OF MENINGEAL WORM ANTIBODIES IN ELK

Introduction

Parelaphostrongylus tenuis, or meningeal worm, is a parasitic nematode with several distinct life stages (Adamson 1986). The normal definitive host for *P. tenuis* is white-tailed deer (*Odocoileus virginanus*). In this relationship, adult meningeal worms produce larvae that are voided with the deer's feces (Anderson 1963, Lankester 2001). A variety of terrestrial gastropods species serve as the meningeal worm's intermediate host and gastropods are presumably infected after contacting the larvae on feces or the soil matrix (Anderson 1963, Lankester and Anderson 1968).

While meningeal worm infections are generally clinically benign in the normal definitive host (Forrester and Lankester 1998), *P. tenuis* infections often detrimentally influence species that serve as abnormal hosts (Anderson 1966, Garner and Porter 1991, Pybus et al. 1992). Abnormal hosts include a wide variety of cervid species, including elk (Anderson et al. 1966, Prestwood and Smith 1969). Clinical signs of meningeal worm infections in elk include adipsia, ataxia, visual impairment, seclusion from the herd, listlessness, and excessive salivation (Carpenter et al. 1973, Olsen and Woolf 1978). Anderson (1968) suggested that abnormal hosts undergo these neurological disorders because *P. tenuis* larvae reach these species' central nervous systems in comparatively high numbers, and spend more time developing within the spinal column.

P. tenuis commonly occurs throughout eastern North America, and is widespread in Kentucky (Prestwood and Smith 1969, Comer et al. 1991, Larkin et al. 2003*a*, Wasel

et al. 2003). Larkin et al. (2003*a*) and Alexy (2004) identified *P. tenuis* infection as a causal factor of mortality in Kentucky's restored elk population, which is consistent with records of previous elk reintroductions in the range of the meningeal worm (Carpenter et al. 1973, Severinghaus and Darrow 1976). Furthermore, Alexy (2004) reported significant *P. tenuis* infection in normal definitive and intermediate hosts throughout the Kentucky elk restoration zone, suggesting that elk have a high likelihood of exposure to the parasite.

Habitat influences the relative density of intermediate hosts (Lankester and Anderson 1968, Raskevitz et al. 1991) due to differing levels of cover, moisture and vegetation regimes (Boag 1985, Suominen 1999). Alexy (2004) found significantly higher total gastropod densities in open herbaceous areas in Kentucky than in forest and edge cover types. Alexy (2004) also demonstrated higher densities of infected gastropods in field habitat, though it was not statistically significant. Because the severity of meningeal worm infection in abnormal hosts is positively correlated with the number of *P. tenuis* larvae ingested (Samuel et al. 1992), cervids that use habitat supporting higher densities of infected gastropods may be at increased risk for meningeal worm infection (Peterson and Lankester 1991, Raskevitz et al. 1991, Whitlaw et al. 1996).

Despite this conceptual link between habitat use and *P. tenuis* infection in cervids, previous studies were not able to evaluate this relationship due to the absence of a reliable antemortem test for *P. tenuis* infection (Welch et al. 1991, McCollough and Pollard 1993, Bienek et al. 1998). This complication has been ameliorated following the development of an enzyme-linked immunoassay (ELISA) that identifies *P. tenuis*

antibodies within blood samples (Ogunremi et al. 1999, Ogunremi et al. 2002*b*). I compared elk habitat use in Kentucky to *P. tenuis* antibody levels to identify if any relationship existed between habitat use and subsequent meningeal worm infection.

Study Area

General.— The elk restoration zone was originally comprised of 14 counties in southeastern Kentucky, but was expanded to 16 counties to facilitate potential movement between Kentucky and Tennessee elk populations (D. Crank, KDFWR, personal communication). This restoration zone lies along the Cumberland Plateau, an area historically characterized by rugged topography covered with continuous second and third growth deciduous forest (Overstreet 1984). Mixed-mesophytic forest association covered most of the richer forested sites, while oak-pine and oak-hickory associations predominated on more xeric locations (Wharton and Barbour 1973). Common overstory species throughout the region include tulip tree (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), red maple (*A. rubrum*), American beech (*Fagus grandifolia*), basswood (*Tilia* spp.), yellow buckeye (*Aesculus flava*), northern red oak (*Quercus rubra*), white oak (*Q. alba*), hemlock (*Tsuga canadensis*), black walnut (*Juglans nigra*), black cherry (*Prunus serotina*), shagbark hickory (*Carya ovata*), and white ash (*Fraxinus americana*) (Wharton and Barbour 1973).

Land use has considerably altered the eastern Kentucky landscape (Maehr et al. 1999), resulting in a mosaic that was approximately 80% second and third growth forest, 10% active and reclaimed surface mines, 9% agricultural or cleared lands, and 1% urban (Cox 2003). Coal extraction through surface mining created herbaceous patches that varied in size, ranging from relatively small openings to 1,200-ha fields (Larkin 2001).

Species commonly found on reclaimed surface mines included Kentucky-31 tall fescue (*Lolium arundinaceum*), bush clover (*Lespedeza* spp.), birds-foot trefoil (*Lotus corniculatus*), crown vetch (*Coronilla varia*), perennial ryegrass (*Lolium perenne*), orchardgrass (*Dactylis glomerata*), black alder (*Alnus glutinosa*), autumn-olive (*Elaeagnus umbellata*), white pine (*Pinus strobus*), and black locust (*Robinia psuedoacacia*) (Larkin 2001).

Hill (1976) described the climate as temperate humid continental, with warm summers and cool winters. Mean annual temperature was 13º C (National Oceanic and Atmospheric Administration 2007), and averaged 117 freeze-free days per year (United States Department of Agriculture Soil Conservation Service 1981). Precipitation averaged 125 cm annually, and was evenly distributed throughout the year (National Oceanic and Atmospheric Administration 2007).

Specific.— The study area was a contiguous block of land, but differences in land management resulted in demarcations between property boundaries (Figure 3.1). The Laurel Fork area encompassed approximately 1,200-ha located in Perry and Breathitt counties, and contained property owned by the University of Kentucky (UK) and International Coal Group. Most of the area was maintained as the Paul Van Booven Wildlife Management Area (WMA) through a cooperative agreement between UK and KDFWR. The Laurel Fork area primarily consisted of recently reclaimed surface mines (< 15 years) and remnant forest patches, though active surface mining occurred on small portions of the peripheral landscape throughout the study.

The Starfire area was an approximately 7,400-ha site located in Perry and Knott counties that consisted primarily of reclaimed surface mines and remnant forest patches;

active surface mining affected a substantial portion of the area during the course of the study. Big Elk Mining Company was the primary property owner. Previous studies refer to this site as the Addington WMA or Cyprus-Amax WMA.

The Beech Fork area comprised approximately 700-ha in Knott County; Kentucky River Properties was the sole property owner. The area consisted primarily of recently reclaimed surface mines (< 5 years), though some remnant forest patches remained. No active surface mining occurred at this location during the study, but large portions of the area underwent reclamation practices during this time.

Methods

Calf data collection.— I captured elk neonates using three different search methods: monitoring cows for parturition behavior, ground-based field searches in traditional calving locations, and aerial searches using a helicopter equipped with an observer using an infrared scope. Calf capture occurred during the months of May and June during 2004-06. Capture techniques followed practices described by White et al. (1972) and Seward (2003).

I considered altered movement patterns, abrupt movement from the herd (Vore et al. 1996, Vore and Schmidt 2001), and a reluctance of lone females to move away in the presence of researchers (Seward 2003) as indicators of parturition behavior. Upon observing any of the aforementioned behaviors I proceeded to walk in a grid pattern throughout the general area in which I viewed the cow. I adjusted search pattern intensity to habitat type and relative cover, spending more time in areas with higher amounts of vegetative cover. I continued in this manner until I either located the calf or decided that I had covered the area sufficiently to locate a calf had one been present.

I identified traditional calving locations through correspondence with Kentucky Department of Fish and Wildlife Resources personnel (K. J. Alexy, D. Crank, and C. Logsdon, KDFWR, personal communication). Calving areas frequently included forest edge, shrub habitat, or pond edges. I conducted ground searches in these areas using the technique described for parturition behavior searches.

I used a Thermal Eye 250D (L-3 Communications Infrared Products, Dallas, TX, USA) handheld infrared scope to locate bedded calves during aerial capture exercises. Helicopters employed in the aerial searches included a 206B Jet Ranger III (Bell Helicopter Textron, Inc., Fort Worth, TX, USA) and an Enstrom 280fx (Enstrom Helicopter Corporation, Menominee, MI, USA). After locating a calf, the observer in the helicopter directed capture efforts to a ground crew via a two-way radio.

I equipped captured elk with expandable VHF radio collars (Advanced Telemetry Systems, Inc., Isanti, MN., USA) that were equipped with a 4-hour, mortality delay switch. I placed one-piece plastic ear tags (Farnam Companies, Inc., Phoenix, AZ, USA) in each calf to facilitate recognition of individuals during tracking efforts. I collected approximately 20 mL of blood from the jugular vein of each calf and recorded neonate sex, approximate age based on umbilicus healing and hoof epithelium wear, Global Positioning System (GPS) location of the capture site, and body weight.

I attempted to recapture each study animal from the 2004 and 2005 cohorts at approximately 6 months of age. KDFWR biologists used JM Standard Rifles (Dan-Inject, Borkop, Denmark) to administer either carfentanil citrate (Wildlife Pharmaceuticals, Inc., Fort Collins, CO, USA) or a Telazol®/xylazine (Fort Dodge Animal Health, Overland Park, KS, USA) mixture to the study animal. All

immobilization drug doses were calculated after Kreeger et al. (2002). Upon recapture I collected a follow-up blood sample and equipped the elk with a permanent radio collar (Advanced Telemetry Systems, Inc., Isanti, MN, USA and Telonics Inc., Mesa, AZ, USA) with a 4-hr mortality delay switch.

Blood sample analysis.— I separated whole blood into serum and red blood cells using a centrifuge, then stored the serum in cryogenic vials at -23º C for subsequent analysis. Prairie Diagnostic Services (Regina, Saskatchewan, Canada) conducted all *P. tenuis* serodiagnostic tests using indirect ELISAs after Ogunremi et al. (2002*b*). Prairie Diagnostic Services designated serum samples as negative, suspect or positive with respect to *P. tenuis* antibody presence based on optical density values. Cutoff values for serum diagnosis were -0.150-0.399 for negative diagnosis, 0.400-0.699 for suspect diagnosis, and 0.700-1.687 for positive diagnosis. All suspect and positive diagnoses fell above the optical density value cutoff for positive diagnosis established by Ogunremi et al. (2002*b*). We considered all suspect diagnoses to be positive.

Radio telemetry.— I intensively monitored collared study animals from May 2004 to August 2007, and obtained locations of elk using a combination of triangulation and homing (Mech 1983). I obtained locations on each animal at least 4 times weekly, and monitored individuals during crepuscular, diurnal and nocturnal periods to gain information about habitat use patterns throughout the day. I attempted to attain a visual confirmation of the study animal when possible. I recorded animal ID, group size, age and sex composition of the group, cover type (herbaceous, edge, shrub, forest and bare ground), behavior, distance to forest edge (m), and slope aspect for each observation. I took a Global Positioning System (GPS) location from a distance sufficient to prevent elk

from fleeing, then took a compass bearing from the GPS point to the animal and measured the distance with a Bushnell (Bushnell Corporation, Overland Park, Kansas, USA) rangefinder. I produced a biangulation point using a minimum of two GPS locations and their associated compass bearings when conditions or terrain prevented visual confirmation of the study animal; I attempted to attain a 90º bearing intersection.

Habitat analysis.— I used Patch Grid extension (Elkie et al. 1999) in ArcMap 9.2 (ArcGIS: Environmental Systems Research Institute, Redlands, CA, USA) and the cover type in which I observed individual elk while conducting telemetry locations to quantify habitat variables for identification of potential differences that elk habitat use could have had on *P. tenuis* infection rates.

Several preparatory steps were necessary before I could employ Patch Grid. The most current land cover map for the elk restoration zone was the Anderson Level II Kentucky Land Cover Data Set 2001 (KLDC-01). Landscape and vegetation regimes can change rapidly in a system exhibiting active surface mining and reclamation. As a result, it was unlikely that habitat classes depicted in KLDC-01 were identical to habitat we observed from 2004-07. I reclassified KLDC-01 into herbaceous, forest, urban and water habitat classes (Table 3.1) to minimize the effects that outdated land cover data may have had on data analysis, and denoted this landcover map KLDC-01 Reclass. I included information from herbaceous and forest habitat classes for Patch Grid analysis; urban and water habitat classes were not included because they were largely absent from the daily movement locations. Patch Grid variables generated from KLDC-01 included mean patch size (MPS) in hectares; edge density (ED), the amount of edge relative to the landscape area (m/ha); and mean core area (MCA), the average size of disjunct core

patches (ha). I chose these variables because they quantify components of edge and field habitat that are hypothesized to be associated with *P. tenuis* infection in elk (Raskevitz et al. 1991).

I calculated mean daily movement from the locations of all individuals from time of capture until May of their birth year. I chose May as the cutoff because it represented the last month in which we collected a blood sample from any individual. I used ArcMap to calculate total meters moved for individuals exhibiting consecutive locations separated by 20-28 hours. I chose this period to incorporate a time buffer in my approximation of mean daily movement. I classified these measurements based on the month in which we collected the location, and calculated the mean distance travelled for individuals that exhibited more than one value in the same month. I calculated mean daily movement to the nearest hundred meter for both sexes for each month (Table 3.2). Daily movement values were similar for males and females in most months, so I chose the largest mean value for each month as the default daily movement. I then isolated telemetry locations gathered for each individual from the date of capture to the location immediately prior to the secondary blood sample collection. I generated a buffer around each individual's locations based on mean daily movement for each month using ArcMap. I then used Patch Grid to extract habitat variables from KLDC-01 Reclass.

I combined Patch Grid habitat statistics from KLDC-01 Reclass with the cover type information gathered during telemetry sessions (Table 3.3) for each individual location (n = 731). I then analyzed these locations with logistic regression (PROC LOGISTIC; SAS Institute Inc. 1998) using forward selection (Appendix C) with an alpha

level of 0.15. I pooled 2004 and 2005 cohort data to increase the sample size of our study animals (n = 35) in relation to number of variables measured (n =11).

Results

The final model for the probability of elk to exhibit meningeal worm antibodies based on habitat use was:

Probability of infection = $-1.49 + 0.22x_1 + 1.17x_2 + 0.62x_3 - 0.01x_4 + 0.01x_5 + 0.01x_6$; where x_1 = ObsHerb, x_2 = ObsShrub, x_3 = ObsBare, x_4 = HerbMCA, x_5 = ForED, and x_6 = ForMCA.

The final model suggested that probability of meningeal worm infection in elk was positively associated with observations in herbaceous cover type, observations in shrub cover type, observations in bare cover type, forest edge density and forest mean core area, and negatively associated with herbaceous mean core area (Table 3.4). Variables not significant to the model were observations in edge cover type, observations in forest cover type, herbaceous mean patch size, herbaceous edge density, and forest mean patch size.

Discussion

The complexity of the *P. tenuis*-cervid relationship can prove challenging when interpreting results. Alexy (2004) found the density of terrestrial gastropods to be significantly higher in field habitat than either edge or forest habitat. Furthermore, Maze and Johnstone (1986) identified reclaimed surface mines as a focus for infected gastropods that could influence transmission of *P. tenuis* to definitive hosts. These

findings correspond to the positive association I detected between observations in herbaceous cover type, but does not account for the negative association between herbaceous mean core area and probability of exhibiting meningeal worm infection. The model also suggested that observations in shrub cover type, observations in bare cover type, forest edge density, and forest mean core area were positively associated with probability of exhibiting *P. tenuis* infection. This combination of significant variables does not correspond to Alexy (2004) research, which noted significantly lower gastropod densities in forest and edge habitat components compared to herbaceous habitat.

Several possible explanations exist for these seemingly anomalous results. First, data used to establish *P. tenuis* infection rate in gastropods noted by Alexy (2004) were not collected concurrently with blood samples used to determine meningeal worm infection in elk. The temporal gap between these data collection periods could prove problematic when attempting to compare the two studies. For instance, Lankester and Anderson (1968) demonstrated that climatic factors could influence development of *P. tenuis* larvae in intermediate hosts and affect gastropod mobility. Furthermore, Whitlaw and Lankester (1994) proposed that environmental factors play the greatest role in determining yearly fluctuations in meningeal worm infection rates of definitive hosts. Because climatic factors fluctuate annually, intermediate host densities and infection rates may have differed from values recorded by Alexy (2004) in the years in which I collected data on elk infection. Given that results from Alexy (2004) reflect gastropod infection data pooled from 2000-02, the effects of this temporal discrepancy may be somewhat mitigated.

Secondly, the landscape variables identified using Patch Grid from KLDC-01 Reclass may be increasingly inaccurate since land use changes constantly altered the study area. As previously noted, Starfire had extensive surface mining and Beech Fork was undergoing reclamation during the study. Variables derived by Patch Grid were based on landscape patterns derived > 3 years prior to the initiation of this study. The constant habitat fluctuations induced by these changes limited my ability to accurately reclassify KLDC-01 to match landscape patterns during the time of this study. This may have reduced the value of Patch Grid parameters used to model *P. tenuis* antibody presence and elk habitat use.

The duration of the sampling period may have also confounded my results. Ogunremi et al. (2002*b*) confirmed positive serodiagnosis of *P. tenuis* antibodies by 28 days post-inoculation in all captive elk experimentally infected with meningeal worm, and some elk maintained antibodies until euthanized 243 days post-inoculation. I began secondary blood sample collection from study animals at approximately 6 months of age, but was unable to obtain samples from some individuals until they were nearly 11 months old. This prevented me from determining when the elk encountered infected gastropods, and consequently diminished my ability to correctly estimate associations between habitat use and presence of *P. tenuis* antibodies. To nullify this problem, blood sample collection would have to occur at much shorter intervals – preferably ≤ 28 days – throughout the course of the study. A blood collection regimen of such intensity would likely prove difficult to achieve under field conditions. Successful implementation of such an intense sample collection would require a correspondingly higher commitment of personnel, time, and funds.

Finally, some of the individuals included in the habitat analysis received maternal meningeal worm antibodies as calves (Chapter 2). The residence time of maternal *P. tenuis* antibodies in elk is unknown, introducing the possibility that these individuals maintained maternal antibodies through the second blood sample collection. If such proved true, these animals should not be included in habitat analysis since they did not form an independent immune response to *P. tenuis* exposure. I was unable to omit these individuals from my habitat analysis, because most (55%) calves received maternal antibodies and removal of these individuals would have reduced sample size to a number too low for statistical analysis of the habitat variables.

My habitat analysis indicated several cover type and habitat factors may be associated with the likelihood of elk developing *P. tenuis* infection, but the biological significance of these results are questionable due to confounding factors. Further research could potentially resolve these issues and generate habitat management prescriptions for reduction of *P. tenuis* infection in elk. Regardless, the Kentucky elk population has increased from 1,541 translocated animals to an estimated 8,500 individuals in 2008 (T. Brunjes, Kentucky Department of Fish and Wildlife Resources, personal communication) despite *P. tenuis* infection. Consequently, habitat management for the sole purpose of decreasing *P. tenuis* infection is likely unnecessary in the Kentucky elk population.

The residence time of maternal meningeal worm antibodies in elk calves should be determined before conducting further research examining relationships between elk habitat use and *P. tenuis* infection (Chapter 2). Future studies investigating *P. tenuis* infection in elk should not focus only on habitat use, since environmental variables

including precipitation (Behrend and Witter 1968, Gilbert 1973, Eveland et al. 1979, Peterson and Lankester 1991, Bogaczyk et al. 1993), mean summer temperature (Peterson et al. 1996), and soil type (Maze and Johnstone 1986) may affect meningeal worm infection in cervids.

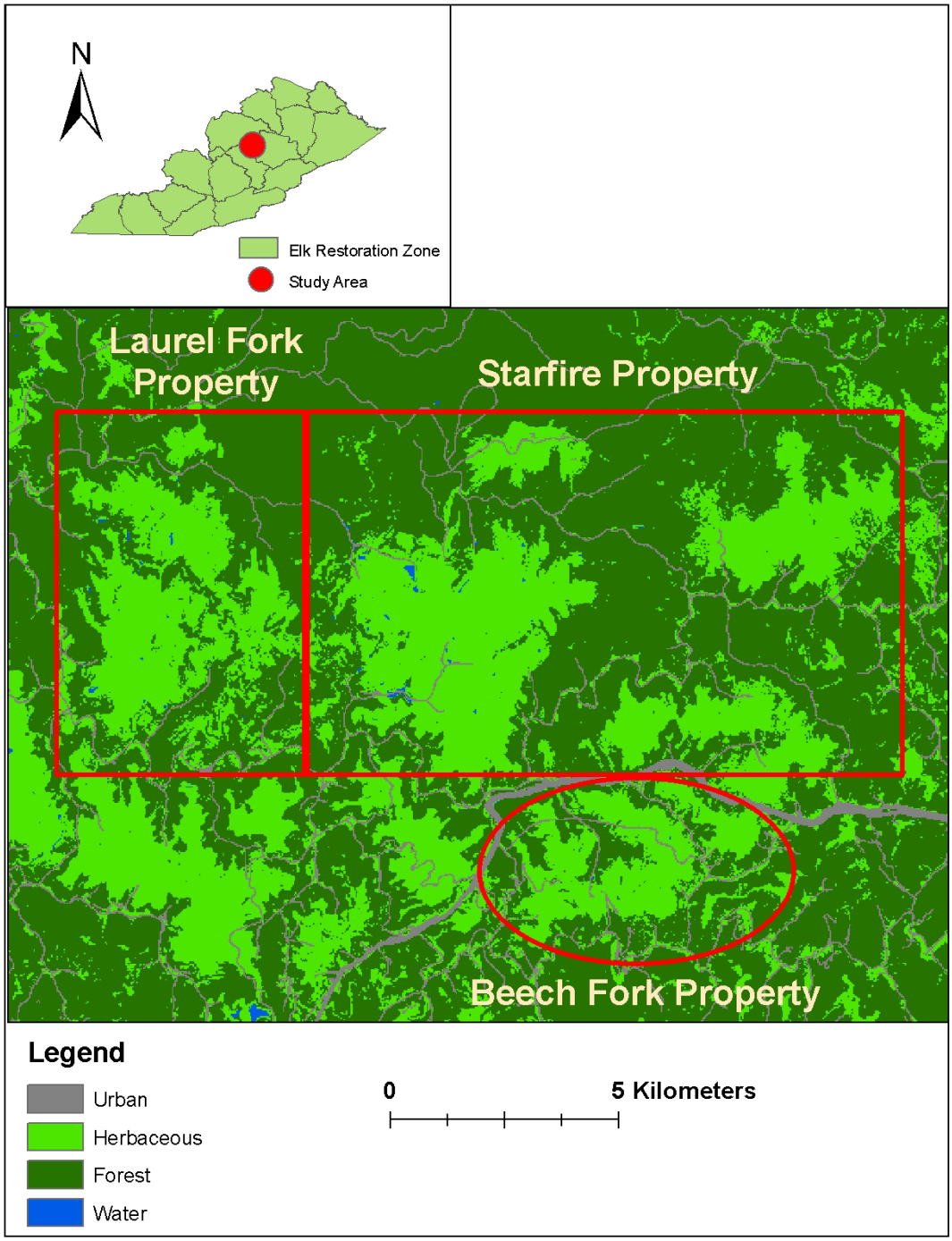

Figure 3.1. Specific study area properties with habitat classes in relation to elk restoration zone.

Table 3.1. Relationships between original KLDC-01 land cover map and KLDC-01 Reclass land cover map.

KLDC-01 Habitat Category	Reclassified Habitat Category
Developed, Open space	Urban
Developed, Low intensity	
Developed, Medium intensity	
Developed, High intensity	
Cropland	Herbaceous
Pasture/Hay	
Herbaceous	
Shrub	
Openland Mined	
Barren	
Mined Bare	
Oak Forest	Forest
Yellow Poplar Forest	
Mixed Deciduous Forest	
Pine Forest	
Red Cedar Forest	
Hemlock Forest	
Oak-Pine Mixed Forest	
Hemlock-Mixed Deciduous Forest	
Other Mixed Forest	
Deciduous Woodland	
Coniferous Woodland	
Mixed Woodland	
Oak/Deciduous Floodplain Forest	
Riparian Forest	

Table 3.1. (Continued)

KLDC-01 Habitat Category	Reclassified Habitat Category
Bald Cypress Wetland Floodplain Forest Woodland Wetland Black Willow Wetland Mixed Shrub Wetland Emergent Wetland	Forest
Water	Water

Table 3. 2. Mean calculated daily movement (m) of elk as a function of sex and month in Kentucky, 2004-2006.

Month	Male		Female	
	Distance traveled (m)	Number of comparisons	Distance traveled (m)	Number of comparisons
May	900	13	800	14
June	1300	8	1100	7
July	1600	8	1400	9
August	900	33	1200	25
September	1400	26	1300	32
October	900	9	900	10
November	1700	13	1500	20
December	1500	30	1200	33
January	1700	32	2000	35
February	900	12	1000	25
March	1200	23	900	43
April	800	3	1200	13
May	1200	9	1300	10

Table 3.3. Parameters considered in logistic regression model comparing *P. tenuis* antibody presence to elk habitat use.

Parameter name	Name and unit of measurement
ObsHerb	Observed location in herbaceous cover type (GPS location)
ObsEdge	Observed location in edge cover type (GPS location)
ObsShrub	Observed location in shrub cover type (GPS location)
ObsFor	Observed locations in forest cover type (GPS location)
ObsBare	Observed location in bare cover type (GPS location)
HerbMPS	Herbaceous mean patch size (ha)
HerbED	Herbaceous edge density (m/ha)
HerbMCA	Herbaceous mean core area (ha)
ForMPS	Forest mean patch size (ha)
ForED	Forest edge density (m/ha)
ForMCA	Forest mean core area (ha)

Table 3.4. Parameters included in logistic regression model comparing *P. tenuis* antibody presence to elk habitat use.

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > Chi-Square
Intercept	1	-1.49	0.24	37.85	<0.01
ObsHerb	1	0.22	0.11	3.81	0.05
ObsShrub	1	1.17	0.17	47.36	<0.01
ObsBare	1	0.62	0.30	4.15	0.04
HerbMCA	1	-0.01	0.01	2.91	0.09
ForED	1	0.01	0.01	4.79	0.03
ForMCA	1	0.01	0.01	7.40	0.01

CHAPTER FOUR

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

The first account of meningeal worm infection in abnormal definitive hosts was a description of "moose sickness" by Thomas and Cahn (1932). The origin of these symptoms eluded researchers until Anderson (1964) identified *P. tenuis* as the causative factor of meningeal worm infection in cervids. This discovery opened the door for subsequent questions concerning the interrelationship between *P. tenuis*, gastropods, white-tailed deer, and meningeal worm's abnormal definitive host species. The premise of parasite-mediated competition between white-tailed deer and other cervids played a central role in much of the ensuing meningeal worm research, leading some investigators to conjecture that *P. tenuis* infection could exclude abnormal host populations throughout the parasite's range (Bergerud and Mercer 1989).

Such sweeping assertions have largely proven inaccurate, however, as the literature contains examples of elk (Raskevitz et al. 1991, Bender et al. 2005), moose (Upshall et al. 1987, Aho et al. 1995), and fallow deer (Davidson et al. 1985) populations persisting for extended periods in the presence of white-tailed deer harboring *P. tenuis*. This capacity for sustained existence of entire populations, when coupled with the ability of individual elk to survive subclinical meningeal worm infection (Samuel et al. 1991) seems to suggest that *P. tenuis* is somewhat ineffective at facilitating parasite-mediated competition. Indeed, the widespread incidence of maternal *P. tenuis* antibody transfer (Chapter 2) implies that elk recruitment continues despite meningeal worm infection. Nevertheless, supposed inefficiency in the *P. tenuis*-definitive host complex does not preclude the existence of parasite-mediated competition. It is possible that the lens of

current research simply cannot distinguish the "complex" but "subtle" associations that characterize parasite-mediated competition (Price et al. 1986).

My inability to identify a clear relationship between elk habitat use and meningeal worm infection likely stemmed from the complexity of these interactions, although potential confounding factors were present (Chapter 3). Identification of a correlation between abnormal definitive host habitat use and *P. tenuis* infection could direct land management efforts that attempt to decrease meningeal worm infection rates in elk. Habitat manipulation to decrease meningeal worm related mortality may prove beneficial in some areas, but *P. tenuis* infection will probably not be the issue of greatest importance to natural resource professionals managing Kentucky elk in the future. High reproductive rates and limited mortality (Larkin et al. 2003*b*) following reintroduction have resulted in strong growth of the Kentucky elk herd across the restoration zone (Dahl 2008). The lack of extant large predators capable of stabilizing population growth delegates sole management responsibility to humans.

Managers should embrace a proactive approach to ensure that the public support observed during the planning stages of the reintroduction effort (Maehr et al. 1999) is not lost as a result of negative human-wildlife interactions. Specific measures could include liberalized harvest rates, programs to increase hunter access to properties with elk, harvest strategies structured to decrease local population numbers, selective removal of nuisance animals, habitat improvement to encourage retention of elk on lands that will minimize nuisance problems, or reintroduction of predator species capable of successfully targeting elk. Such measures, conducted with suitable stakeholder input,

could promote efficient management of this growing elk population while fostering a spirit of cooperation between KDFWR and the public.

Appendix A

SAS code for Fisher's exact test used to analyze elk neonate sex and association with acquisition of maternal *P. tenuis* antibodies.

```
PROC FREQ;
Weight Count;
Tables Sex*Diagnosis / CHISQ;
RUN;
```

Appendix B

SAS code for logistic regression used to test for association between elk predicted birth weight and transfer of maternal *P. tenuis* antibodies.

```
PROC LOGISTIC DESCENDING;
MODEL Diagnosis = PWeight /
SELECTION = FORWARD SLENTRY = 0.15 SLSTAY = 0.15 DETAILS;
RUN;
```

Appendix C

SAS code for logistic regression used to test for associations between habitat variables and *P. tenuis* infection in elk.

```
PROC LOGISTIC DESCENDING;
MODEL Diagnosis = ObsHerb ObsEdge ObsShrub ObsFor ObsBare HerbMPS HerbED
        HerbMCA ForMPS ForED ForMCA /
SELECTION = FORWARD SLENTRY = 0.15 SLSTAY = 0.15 DETAILS;
RUN;
```

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